CHANGES IN CONCENTRATION OF THIOL GROUPS IN THE PROPHASE OF MITOSIS OF NORMAL AND TUMOR CELLS

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The content of SH- and SS-groups and the SH/SS ratio in prophase (the period of "assembly" of the mitotic apparatus) undergo different changes in normal and tumor cells. In normal cells (human amnion) the number of thiol groups shows a marked increase, the content of SH-groups being much greater than that of SS-groups. In cells of Ehrlich's carcinoma, the concentration of thiol groups changes only slightly and the SH/SS ratio falls by almost half. In the cells of an 8-day malignant glioma, the number of thiol groups in prophase increases sharply, the SH/SS ratio approximating to 1, while in a 16-day tumor, in contrast to other types of long-growing cells in the body, this ratio rises.

One of the problems in the physiology of mitosis which has attracted most attention is that of the chemical nature of the links between contractile proteins forming the mitotic apparatus. The observations of Mazia [11,12] show that "assembly" of the mitotic apparatus takes place by the formation of disulfide bridges between macromolecules. Our experiments on a culture of human amnion cells confirmed these views and showed that after treatment with thiol preparations (p-chloromercuribenzoate, cysteine hydrochloride) delay in division occurs in metaphase and many pathological mitoses appear, resulting from disturbance of formation of the mitotic apparatus and from abnormalities of separation of the chromosomes [3].

Further observations showed that, in contrast to normal cells, procedures directed at the thiol mechanism of mitosis in tumor cells (primary culture of malignant glioma and cells of the ascites strain of Ehrlich's carcinoma) gave rise to a paradoxical effect. The number of pathological mitoses and metaphase delay after treatment with thiol compounds not only were not increased, but on the contrary, were decreased, leading to partial recovery of the normal mitotic regime [2,4]. The results of these experiments suggested that the thiol mechanism of assembly of the mitotic apparatus is disturbed in tumor cells, and that it is these disturbances which are responsible for the appearance of pathological mitoses typical of tumor cells.

To test this hypothesis, the present investigation was carried out, with the object of studying one of the manifestations of the thiol mechanism of assembly of the mitotic apparatus: changes in the number of thiol groups in the prophase of mitosis compared with their number in the interphase of normal and tumor cells.

According to Mazia's hypothesis [11,12], the assembly of the mitotic apparatus consists of the conversion of intramolecular disulfide bonds into intermolecular. Chemically speaking, this mechanism is expressed as cyclic changes (the "Rapkine cycle" [3,14]) in the content of sulfhydryl groups in the course of mitosis, reciprocal relationships between their content in the proteins of the mitotic apparatus and proteins (polypeptides) of the trichloroacetic extracts, and also an increase in the content of thiol groups in prophase [11,12,16]. The increase in the number of SH-groups in prophase, when assembly of the mitotic apparatus takes place, has been studied not only biochemically, but also by cytochemical methods in sea urchin's eggs [10], HeLa cells [17], and lily pollen [15].

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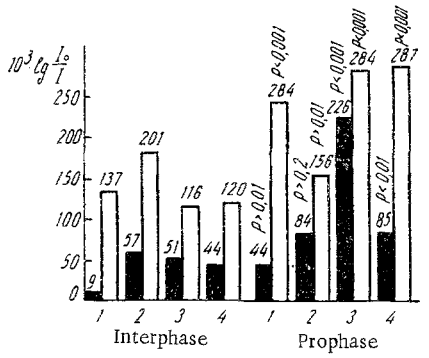

Fig. 1. Concentration of thiol groups in interphase and prophase. From left to right: amnion cells (1), cells of Ehrlich's ascites carcinoma (2), cells of a primary culture of glioblastoma from tumors grown in vivo for 8 (3) and 16 (4) days. Unshaded columns represent SH-groups, shaded columns SS-groups.

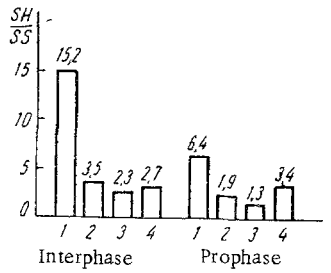

Fig. 2. Ratio between concentrations of SH- and SS-groups in interphase and prophase. Legend as in Fig. 1.

EXPERIMENTAL METHOD

Experiments were carried out on monolayer cultures of human amnion cells and primary cultures of a malignant glioma (mouse glioblastoma, strain 51/11 of Yablonovskaya), and also on films of Ehrlich's ascites carcinoma cells from mice. The primary glioblastoma culture was obtained from tumors grown in vivo for only 8-10 days and also from tumors grown in vivo for 14-16 days. The 3-day cultures and films were fixed in 96° alcohol or in Carnoy's fluid. To detect SH- and SS-groups, the reaction of Barrnett and Seligman [7] was carried out with 2,2'-dioxy-6,6'-dinaphthyldisulfide (DDD) followed by staining with Fast black (Fast black salt, 99.1%, England). The content of SH- and SS-groups was determined quantitatively by cytophotometry [8,9,17,18].

The cytophotometric measurements were made by a photoelectric method on a single-beam probe cytophotometer [1,5,6]. The diameter of the probe beam of light in the focal plane was 1 μ. The optical density was determined as the logarithm of nontransparency in the yellow-green region of the spectrum (530 mμ) from the formula $E = \log \frac{\Phi_0}{\Phi}$. Absorption spectra of structures of different density were first obtained. These measurements showed no changes in the spectral properties of the dye with an increase in its concentration, so that it could be considered as a suitable dye for cytophotometry. The mean optical density of the cytoplasm was calculated from measurement of the optical density of the cytoplasm of 50 interphase cells and 10 prophase cells from each of the objects. The dimensions of the structures were ignored, because within the limits of each object the mean size of the cells was the same in specimens stained for SH- or SS-groups. For this reason, within the limits of each object, the optical density of a particular structure reflected changes not only in concentration, but also in absolute content of the dye.

When determining the content of SS-groups, appropriate corrections had to be introduced for their true content. This was because, in order to detect disulfide groups in the specimen, the SH-groups were first blocked with monochloroacetic acid, and the SS-groups present were then converted into SH-groups by means of unithiol. Analysis of control specimens treated with monochloroacetic acid alone showed that a considerable number of SH-groups remained unblocked. Further photometry of the combined reaction for sulfhydryl and disulfide (SH+SS) groups revealed that in all objects the same percentage of SH-groups remained unblocked, and this was allowed for during quantitative estimation of the SS-groups.

EXPERIMENTAL RESULTS

The results of the measurements showed (Fig. 1) that in the prophase of mitosis of human amnion cells, as in other normal cells, there is a marked increase in the concentration of thiol groups. In prophase, just as in interphase, the concentration of SH-groups remains much higher than that of SS-groups.

Different changes take place in the period of "assembly" of the mitotic apparatus in tumor cells. In the cells of Ehrlich's carcinoma in prophase not only did the concentration of SH-groups not increase but, on the contrary, it actually fell slightly. The concentration of SS-groups increased slightly, while the SH/SS ratio fell by almost half. These changes were probably associated with the oxidation and reduction of disulfide bonds during "assembly" of the mitotic apparatus.

In the cells of the malignant glioma, this mechanism showed changes of a different type. As in the amnion cells, the concentration of SH-groups increased sharply. However, unlike in the normal cells, this was accompanied by a smaller increase in the concentration of SS-groups and by a more marked change in the SH/SS ratio.

Changes in the concentration of thiol groups in the period of assembly of the mitotic apparatus were not confined to an increase in concentration of SH-groups, but (contrary to Mazia's hypothesis) the SH/SS ratio was also changed. In amnion cells, this ratio (Fig. 2) fell during prophase by more than half. These changes are probably connected with the oxidation and reduction of disulfide bonds during assembly of the mitotic apparatus. In tumor cells in prophase the ratio between the concentration of thiol groups also fell, but in the malignant glioma grown in vivo for 8 days the changes in concentration of thiol groups were so great that their ratio came close to 1. In the cells of a glioma grown in vivo for a long period, in contrast to other types of cells, the SH/SS ratio was not decreased but, on the contrary, increased.

The results of measurements of the concentration of thiol groups in the period of assembly of the mitotic apparatus thus showed that the concentration of SH- and SS-groups undergoes different changes in normal and tumor cells. It may be postulated that these changes reflect disturbances of the normal thiol mechanism of assembly of the mitotic apparatus in tumor cells. Together with previous experimental results [3,4], these observations help to connect the appearance of pathological mitoses in tumor cells with changes in the thiol mechanism of assembly of the mitotic apparatus.

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